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2-PHENYLETHANOL IN THE LEAVES OF *BURSERA VELUTINA* BULLOCK (BURSERACEAE)

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ABSTRACT

The volatile composition of the leaves of *Bursera velutina* Bullock (Burseraceae) was determined by a gas chromatograph-mass spectrometer (GC–MS). The major component found was 2-phenylethanol (29.5%). This is the first report of 2-phenylethanol in the leaves of a species of the genus *Bursera*. In addition, *B. velutina* also produces monoterpenes, sesquiterpenes, diterpenes and alkanes, making it a species with one of the most complex chemical compositions in the genus. This diverse and unique blend of compounds may play an important role in plant defense against its herbivores.

Key words: Burseraceae, *Bursera velutina*, 2-phenylethanol, Mexico, terpenes.

RESUMEN

Se identificaron los compuestos químicos volátiles presentes en las hojas de *Bursera velutina* usando cromatografía de gases y espectrometría de masas. El compuesto de mayor abundancia fue 2-feniletanol (29.5%). Esta es la primera vez que se reporta la presencia de esta substancia en las hojas de plantas del género *Bursera*. Además de este compuesto aromático, *B. velutina* produce monoterpenos, sesquiterpenos, diterpenos y alcanos, lo que la convierte en una de las especies de mayor complejidad química en el género. Esta combinación diversa y distinta de compuestos podría jugar un papel importante en la defensa contra sus herbívoros.

Palabras clave: Burseraceae, *Bursera velutina*, 2-feniletanol, México, terpenos.

INTRODUCTION

The genus *Bursera* Jacq. ex L. (Burseraceae) includes about 100 species of trees and shrubs native to tropical regions of New World, from the southwestern United States to Peru. *Bursera*'s close Old World relatives are the source of frankincense (*Boswellia*) and myrrh (*Commiphora*). The genus is highly diverse in the tropical dry forests of Mexico where, with about 85 species, it is one of the predominant woody taxa (Rzedowski & Kruse, 1979; Becerra & Venable, 1999; Becerra, 2003). *Bursera velutina* is a narrow endemic that inhabits the warmest areas at low altitudes on the west side of the Balsas River Basin in Southern Mexico. No previous phytochemical characterization has been reported for the leaves of this species.

Bursera produces an array of terpenes, mostly mono- and sesquiterpenes, and alkanes (Evans et al., 2000; Becerra et al., 2001; Evans & Becerra, 2006; Noge & Becerra, 2009; Noge et al., 2010). These compounds are toxic or repellent to herbivorous insects, and in *Bursera* they decrease the survival and growth of their specialized herbivores, the chrysomelid genus *Blepharida* (Becerra, 1994; Becerra et al., 2001). The impact of *Blepharida* on *Bursera* often depends on the defensive status of the plants, and individuals with relatively low concentration of terpenes can be completely defoliated by these beetles (Becerra, 1993).

Blepharida beetles show a preference for colonizing chemically similar plants that are not necessarily phylogenetically close (Becerra, 1997). This preference for chemically similar plants might impose selective pressures on plants promoting divergent chemical components (Becerra, 2007). In this study, we investigated the volatile chemical composition of the leaves of *B. velutina*.

MATERIALS AND METHODS

Plant materials

Samples of leaves of *B. velutina* were collected from mature individuals in natural populations growing in the vicinity of Altamirano, Guerrero between 100–102° W and 17–18° N. Voucher specimens are deposited at the University of Arizona Herbarium (ARIZ) under the number Becerra and Venable 377.

Sample preparation and chemical analysis

Fresh leaves (28-76 mg) of mature individuals were collected and immediately extracted in 2 ml of dichloromethane at 4 °C for more than 24 h. The extracts were then collected into a new glass vial and kept at 4 °C until chemical analysis. The dichloromethane extract of *B. velutina* was mixed with the same amount of dichloromethane containing 10 ng/µl 1-dodecene as an internal standard. Then, 1 µl of the mixture was subjected to GC and GC–MS analyses. The volatile components were identified and the yield of essential oil per weight of leaf tissue was determined. The analyses were replicated 15 times using leaf extracts from different leaves from 8 individuals.

GC–MS analysis was carried out by an Agilent 6890N gas chromatograph linked to an Agilent 5975B mass spectrometer operated at 70 eV using a HP-5MS capillary column (Agilent Technologies, 30 m × 0.25 mm i.d., 0.25 µm film thickness) with helium carrier gas at 1.2 ml/min in splitless mode. The oven temperature was held at 40 °C for 4 min and programmed to increase at 8 °C/min from 40 °C to 240 °C and finally held at 240 °C for 5 min. The injector temperature was maintained at 200 °C and the detector temperature at 280 °C. All compounds except for sabinene were identified by comparing their GC retention times and mass spectra with those of authentic standards. Sabinene was tentatively identified by comparison of the mass spectrum with that of libraries (Wiley7 and NIST05).

GC analysis was performed on an Hewlett-Packard 5890 gas chromatograph with a flame ionization detector, using a DB-5 capillary column (J & W Scientific, 15 m × 0.32 mm i.d., 0.25 µm in film thickness) under the same conditions as those used for GC–MS analysis.

The dichloromethane extract of *B. velutina* was concentrated by evaporating the solvent, and then the yield of the oil of *B. velutina* was calculated.

RESULTS AND DISCUSSION

The yield of oil extracted by dichloromethane from fresh leaves was 4.5 ± 0.6% (w/w). The leaf of *B. velutina* is a rich source of volatile oil similar to the one of *Bursera chemapodicta* (5.3%, Evans & Becerra, 2006) and the confamilial species, *Boswellia sacra* (5.5%, Al-Harrasi & Al-Saidi, 2008). Chemical analysis indicated that 2-phenylethanol (29.5%), α-phellandrene (28.8%) and β-phellandrene (11.0%) were the most abundant compounds in the leaves of *B. velutina* (Table 1). Other terpenes and heptane and its derivatives that are already reported from other *Bursera* species were also found (Evans et al., 2000; Becerra et al., 2001; Evans & Becerra, 2006). This is the first identification of an aromatic compound present in relative large amount in the leaves of a member of the genus *Bursera* (4.8–6.5 mg/g leaf).

Table 1. Volatile composition in the leaf of *Bursera velutina* (N = 15).

Compound	Retention time (min)[a]	Composition (Average ± SE, %)[b]
Heptane	4.00	0.5 ± 0.3
2-Heptanone	6.50	0.3 ± 0.3
2-Heptanol	9.10	0.2 ± 0.1
α-Pinene	10.26	0.9 ± 1.3
Sabinene	11.30	2.6 ± 0.3
α-Phellandrene	12.06	**28.8 ± 3.9**
p-Cymene	12.54	4.2 ± 0.7
β-Phellandrene	12.71	**11.0 ± 1.1**
2-Phenylethanol	14.58	**29.5 ± 4.4**
β-Caryophyllene	20.07	1.8 ± 0.8
Germacrene D	21.82	trace
Phytol	30.59	5.3 ± 1.1

[a]Retention times are based on GC analysis with DB-5MS capillary column.
[b]Percentages are based on GC-FID peak area. Total percentage <100% are due to the presence of minor unidentified compounds. Percentages higher than 10% are bolded trace, <0.1%.

Some aromatic compounds such as guaiacol (0.3%), *p*-cresol (0.2%), vanillin (0.2%) and 2-methoxy-5-methylphenol (0.1%) were detected in the roast aroma extract of *B. graveolens* as minor components (Yukawa et al., 2006).

Two monoterpenes (limonene, 15.7%; α-terpineol, 10.7%) and two sesquiterpenes (spathulenol, 12.5%; β-eudesmol, 12.9%) have been identified as major components of the bark extract of *B. velutina* together with 17 other components (Zúñiga et al., 2005). We detected none of these compounds in the leaves of *B. velutina*. Evans and Becerra (2006) showed that the chemical composition of *B. chemapodicta* was different between leaves and twigs. Thus, tissue-specific localization or production of resin components may not be unusual in the genus *Bursera*.

The chemical components found in the *Bursera* leaves can be classified into three groups based on their biosynthetic pathways (Table 2). Terpenes are further divided into three subgroups based on the number of isoprene units and their origin (Davis & Croteau, 2000). 2-phenylethanol is synthesized from phenylalanine in plants (Watanabe et al., 2002; Tieman et al., 2006), and this pathway is fundamentally different from that of terpenes and short-chain aliphatic alkanes. Leaf components

Table 2. Comparison of the volatile leaf compositions of ten *Bursera* species.

	Composition (%)				
	Aromatic compounds	Terpenes			Short-chain aliphatic alkanes and their derivatives
		Monoterpenes	Sesquiterpenes	Diterpenes (Phytol)	
B. velutina	29.5	47.5	1.8	5.3	1.0
B. schlechtendalii[ab]	0.7	50.7	7.5	1.7	43.5
B. fagaroides purpussi[b]	trace	79.0	20.2	trace	trace
B. linanoe[c]	–	57.6	41.1	–	–
B. ruticola[b]	–	43.9	55.3	–	–
B. biflora[ab]	trace	34.5	31.5	6.3	3.5
B. mirandae[b]	trace	24.6	59.3	–	9.9
B. excelsa[b]	trace	12.4	87.5	–	trace
B. copallifera[b]	–	1.2	94.6	–	trace
B. chemapodicta[bd]	0.6	–	3.9	0.6	93.2

Not detected: –
References: [a]Evans et al., 2000; [b]Noge & Becerra, 2009; [c]Noge et al., 2010, [d]Evans & Becerra, 2006.

of most *Bursera* species generally include only two or three groups, such as monoterpenes, sesquiterpenes and/or alkanes. *Bursera velutina*, however, produces more complex blend of compounds that include at least five different basic pathways.

2-phenylethanol is known to be a floral fragrance in some plants, having a rose-like odor (Knudsen et al., 1993) and attracting various kinds of insects to flowers, such as the cabbage looper moth, *Trichoplusia ni* (Haynes et al., 1991), the cabbage butterfly, *Pieris rapae* (Honda et al., 1998), the long-legged chafter, *Hoplia communis* (Imai et al., 1998) and the lacewing, *Chrysopa carnea* (Zhu et al., 2005). These flower-visiting insects could act as pollinators. This compound has also been known to inhibit the growth of fungi (Lester, 1965; Terenzi & Storck, 1969) and bacteria by breaking down cell membranes and inhibiting DNA synthesis (Berrah & Konetzka, 1962; Slepecky, 1963; Silver & Wendt, 1967). In terms of anti-herbivore defense, 2-phenylethanol has been identified as a feeding deterrent against pine weevil, *Hylobius abietis*, from the non-host plants, *Ilex aquifolium* and *Populus tremula* (Eriksson et al., 2008). Thus, this is a multifunctional compound that can play an array of important roles in plants.

Besides having a relatively complex chemical composition, producing a high concentration of 2-phenylethanol makes *B. velutina* chemically unique, different from other *Bursera* species. We have analyzed the chemical compositions of 65 of the ~100 species in the genus and only a handful of them produce aromatic compounds and only in trace amounts (Becerra, 2007). Furthermore, all of the other *Bursera* species that inhabit the distribution area of *B. velutina*, such as *B. trimera*, *B. kerberii*, *B. trifoliolata*, *B. coyucensis* and others have very dissimilar chemical compositions to that of *B. velutina* (Becerra, 2007). Thus, *B. velutina* is more unlikely to be attacked by their herbivores. For example, neither *Blepharida lineata* that attacks *B. trimera*, *Blepharida pallida* that attacks *B. coyucensis*, nor *Blepharida sparsa* that feeds on *B. kerberii* will accept *B. velutina* as food (personal observation by J.X.B.). *Blepharida flavocostata* feeds on *B. velutina* in the field, but not on the above four sympatric *Bursera* species (Becerra, 2004). *Blepharida* beetles prefer chemically similar *Bursera* species as has been shown for the host recognition of *Pieris napi macdunnoughii* (Lepidoptera: Pieridae). Both larvae and adults of that crucifer specialist accept two naturalized weeds whose leaf glucosinolate profiles are chemically similar to those of preferred native food plants even the weeds are unfavorable for larval development to pupation (Rodman & Chew, 1980). Thus, producing high amounts of 2-phenylethanol may constitute a strong divergence from the sympatric *Bursera*, making *B. velutina* chemically distinct and thereby helping avoid herbivory by unadapted *Blepharida* beetles. Developing unique blends of compounds has also been observed in other burseras. For example, in *B. chemapodicta* the typical terpene resin composition has been completely replaced with heptane and other hydrocarbon derivatives, which has allowed this species to deter herbivory from *Blepharida schlechtendalli*, the specialist of the sympatric sister species *B. schlechtendalli* (Evans & Becerra, 2006).

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